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Short communication

A Newly Recorded Sea Cucumber of the Genus *Psolus* (Holothuroidea: Dendrochirotida: Psolidae) from the East Sea of Korea

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ABSTRACT

A sea cucumber was collected from Gonghyeonjin in the East Sea of Korea at a depth of 50 m on 22 June 2011 and was identified as *Psolus phantapus* (Strussenfelt, 1765). This species belongs to the family Psolidae of the order Dendrochirotida based on morphological characteristics and mitochondrial cytochrome c oxidase subunit I sequence analysis. *Psolus phantapus*, which widely distributes in the Arctic and North Atlantic Oceans, is newly recorded in the Korean fauna. Two *Psolus* species including the previously reported *P. squamatus* are recorded in the East Sea of Korea.

Keywords: *Psolus phantapus*, morphological characteristics, SEM, mitochondrial COI sequence, molecular identification

INTRODUCTION

Sea cucumbers of the family Psolidae have distinctive morphological characteristics compared with other families of the order Dendrochirotida. The dorsal surface has a continuous covering of imbricated calcified scales, and the ventral sole is soft, demarcated and arranged with rows of tube feet along the margin (Deichmann, 1941). The latter characteristic indicates that the specimen attaches itself to a hard substrate. The family Psolidae comprises six genera: *Ceto* Gistel, 1848; *Ekkentropelma* Pawson, 1971; *Lissothuria* Verrill, 1867; *Neopsolidium* Pawson, 1964; *Psolidium* Ludwig, 1887; and *Psolus* Oken, 1815 (see Paulay, 2017), of which the genus *Psolus* has been reported from Korea waters by previous papers (Rho and Shin, 1986; Shin and Rho, 1996; Shin, 2012). The genus *Psolus* comprises 58 species and represents the most successful and widespread genus of the family Psolidae (Paulay, 2017). Species of the genus *Psolus* occur in tropical seas as well as in Arctic and Antarctic seas (Pawson, 1964).

DNA barcoding sequence variation in an approximately 650-bp region of the mitochondrial cytochrome c oxidase subunit I (mt-COI) gene is a powerful tool for identification and discovery of species (Hebert et al., 2003; Ratnasingham and Hebert, 2007), and the region of mt-COI sequence is validated as an effective tool for species discrimination in echinoderms (Ward et al., 2008; Hoareau and Boissin, 2010; Layton et al., 2016). Over 16,000 mt-COI sequences from echinoderms have been deposited in GenBank, providing useful data for various research studies and molecular identification of species. Ninety-eight mt-COI sequences from seven species of the genus *Psolus* are registered in GenBank, and have been used for DNA barcoding of echinoderms (Corstorphine, 2010) and Antarctic echinoderm diversity analysis (O'Loughlin et al., 2011).

A sea cucumber was collected with a fishing net at a depth of 50 m in waters adjacent to Gonghyeonjin of Gangwon-do in the East Sea of Korea on 22 June 2011, and was preserved in 95% ethyl alcohol. Its important morphological characteristics were photographed using a digital camera (G12; Canon, Tokyo, Japan). Ossicles were extracted from small pieces of the dorsal wall, sole, and tube feet, using an NaClO solution (Shin, 2012), and were photographed with stereo and light microscopes (Nikon SMZ 1000, Nikon Eclipse 80i; Tokyo,

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Japan) as well as with a scanning electron microscope (JSM-microscopes 6510; JEOL, Tokyo, Japan). The traditional taxonomic characters used for morphological description as described by Jaeger (1833) and Mortensen (1927) were applied to confirm identification of the specimen. The collected specimen was deposited in the Marine Echinoderm Resource Bank of Korea (MERBK), Sahmyook University, Seoul, Korea.

Total genomic DNA was extracted from gonad tissue following the DNeasy kit protocol (Qiagen, Hilden, Germany). The partial sequence of the mt-COI gene was amplified using a pair of primers conserved in echinoderms, LCOech1aF1 (5′-TTTTTTCTACTAAACACAAGGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al., 1994; Layton et al., 2016). PCR amplification reactions were performed according to Lee (2011). PCR product quality was assessed using a NanoDrop 1000 (Thermo Scientific, Waltham, MA, USA), and PCR products were sequenced using ABI Big Dye Terminator kits (Applied Biosystems, Foster City, CA, USA) on a ABI 3730XL DNA Analyzer. Pairwise estimates of intra- and interspecific divergence were calculated with mt-COI sequences of *Psolus* species recorded at NCBI using the Kimura-2 parameter (K2P) distance model (Kimura, 1980).

Previously, *Psolus squamatus* was recorded from the East Sea of Korea (Rho and Shin, 1986; Shin and Rho, 1996; Shin, 2012), and in the present study, a new *Psolus* species was reported in the East Sea. Thus, two *Psolus* species were recorded from the East Sea of Korea, and a key for the Korean *Psolus* species was prepared.

SYSTEMATIC ACCOUNTS

Class Holothuroidea de Blainville, 1834
Order Dendrochirotida Grube, 1840
Family Psolidae Bruimeister, 1837
Genus *Psolus* Oken, 1815

Key to the species of Genus *Psolus* in Korea

1. Body large, high and vaulted. Sole with three rows of tube feet along margin. Calcified scales of dorsal side small ····· *P. phantapus*
− Body small, almost flat. Sole with two rows of tube feet along margin. Calcified scales of dorsal side large ····· ····· *P. squamatus*

[1*]*Psolus phantapus* (Strussenfelt, 1765) (Fig. 1A−J)

Holothuria phantapus Strussenfelt, 1765: 263, pl. 10; Müller, 1788: 54.
Psolus phantapus Jaeger, 1833: 21; Gray, 1848: 9; Stimpson, 1853: 16; Lütken, 1857: 12; Selenka, 1867: 342; Bell, 1882: 646; Lampert, 1885: 116; Mortensen, 1927: 415; Hansson, 2001: 348; Paulay, 2017: 124710.
Psolus granulatus Grube, 1840: 38.
Psolus laevigatus Ayres, 1851: 63.
Psolus regalis Verrill, 1866: 353; Bell, 1882: 646.

Material examined. One specimen, Gonghyeonjin, Gangwon-do, Korea, 22 June 2011, at 50 m deep with a fishing net.

Description. Body rather high, vaulted and curved to mouth and anus (Fig. 1A). Mouth especially developed into a long, conical, tail-like prolongation (Fig. 1C). Body wall thick, scales of dorsal side rather small (length = 1.0−2.8 mm, width = 0.7−2.0 mm) and somewhat granulated. Ventral sole rather small (length = 7.2 cm, width = 4.3 cm), rectangular, narrower than body width. Tube feet in mid-radius with rudimentary series but with obvious three rows along margin (Fig. 1B). Calcareous ring low, with broad anterior radial teeth and more pointed anterior interradial teeth, with undulated posterior margin (Fig. 1D). Polian vesicle single and stone canal small, embedded in dorsal mesentery. Respiratory trees well developed. Ossicles of dorsal side spherical or oval form with rather complicated structure, grouped into two types (Fig. 1E−H): large (Fig. 1E, G) and small ossicles (Fig. 1F, H). Ossicles of tentacles with irregularly formed perforated plates having six to twenty pores (Fig. 1I). Ossicles of tube feet with small and perforated roundish plates having three to five pores (Fig. 1J).

Size. Body: length = 9.9 cm, width = 5.2 cm.

Color. Body color is light brown in alcohol.

Distribution. Korea (East Sea), Okhotsk Sea, Alaska, Arctic Ocean, Barents Sea, North Sea, White Sea, North Atlantic (Canada, Denmark, Ireland, United Kingdom).

Remarks. *Psolus phantapus* is widespread in the Arctic and North Atlantic Oceans (Mortensen, 1927; Hansson, 2001; Paulay, 2017), and inhabits stones or muddy gravels at 10 to more than 200 m depth in the British Isles (Mortensen, 1927; Southward and Campbell, 2006). The Korean specimen was collected from Gonghyeonjin in the East Sea, at 50 m depth with a fishing net, and seems to inhabit the northern part of the East Sea, which is influenced by the subarctic Liman current. This species is distinguished clearly from *P. squamatus* which is previous recorded species in Korea. Body length of *P. phantapus* (9.9 cm) is twice or three times as large as *P. squamatus* (3.0−5.2 cm). *Psolus squamatus* has large calcified scales in dorsal side (length = 4.4−6.6 mm,

Korean name: [1*]갈색비늘해삼(신칭)

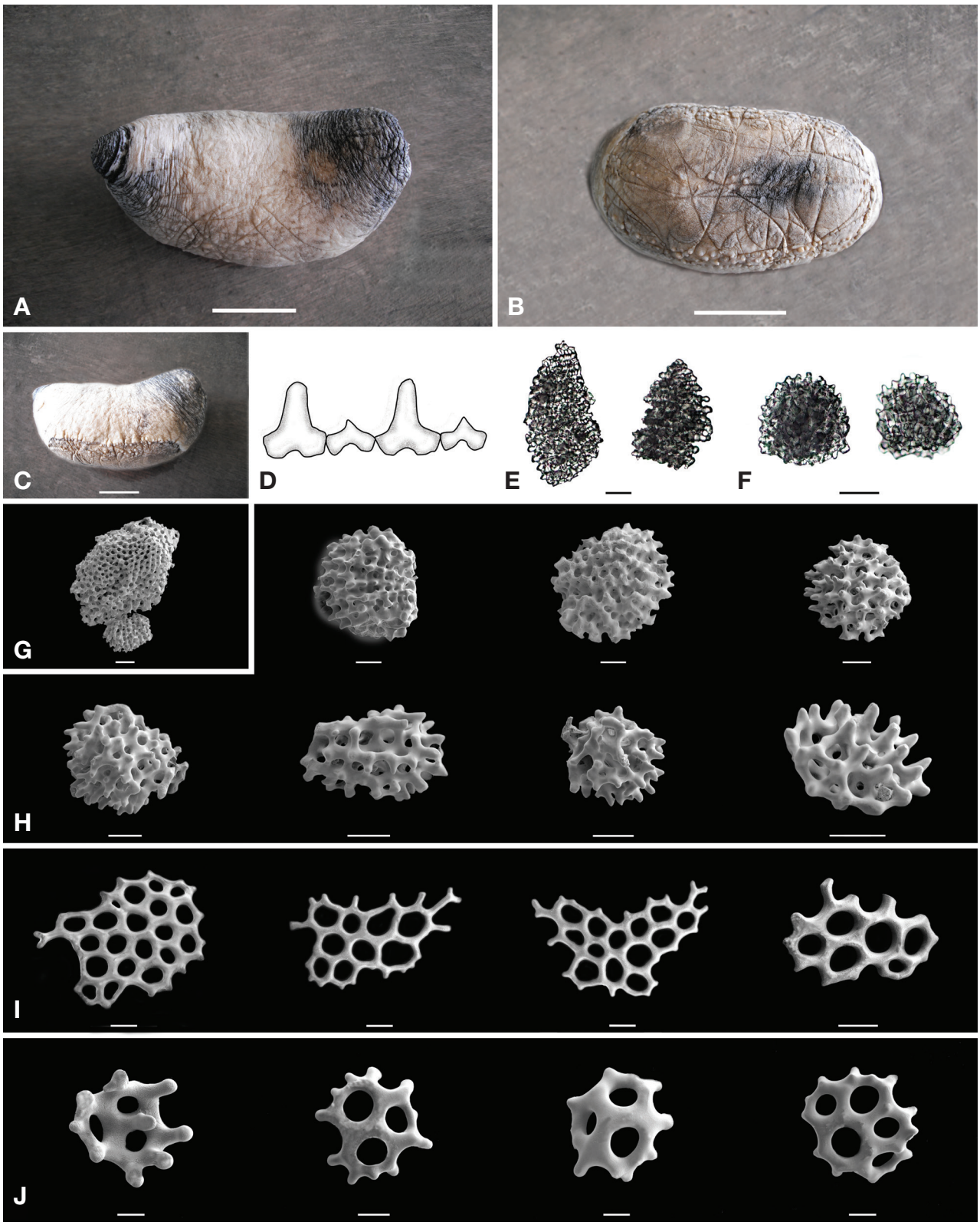

Fig. 1. *Psolus phantapus*, scanning electron microscope photographs (G–J). A, Dorsal view; B, Ventral view; C, Lateral view; D, Calcareous ring; E, G, Large ossicle of dorsal body wall; F, H, Small ossicle of dorsal body wall; I, Perforated plate of tentacle; J, Small perforated plate of tube feet. Scale bars: A–C = 2.5 cm, E–G = 100 μm, H = 50 μm, I, J = 20 μm.

Table 1. Inter- and intra-specific pairwise distance values for genus *Psolus* species, calculated using the K2P distance model, based on partial sequences of the mitochondrial COI gene

Species	1	2	3	4	5	6	7	8	9	10	11	12	13
1. *P. phantapus* (Korea; MF347379)													
2. *P. phantapus* (Russia; GU672427)	0.000												
3. *P. phantapus* (Russia; GU672428)	0.000	0.000											
4. *P. phantapus* (Canada; HM542350)	0.000	0.000	0.000										
5. *P. phantapus* (Canada; HM543062)	0.002	0.002	0.002	0.002									
6. *P. phantapus* (Canada; HM543063)	0.002	0.002	0.002	0.002	0.005								
7. *P. phantapus* (Canada; HM543066)	0.000	0.000	0.000	0.000	0.002	0.002							
8. *P. antarcticus* (Antarctica; HM196613)	0.207	0.207	0.207	0.207	0.204	0.204	0.207						
9. *P. charcoti* (Antarctica; HM196655)	0.237	0.237	0.237	0.237	0.233	0.233	0.237	0.240					
10. *P. chitonoides* (Canada; HM542342)	0.105	0.105	0.105	0.105	0.105	0.102	0.105	0.207	0.230				
11. *P. dubiosus* (Antarctica; HM196601)	0.220	0.220	0.220	0.220	0.217	0.217	0.220	0.070	0.220	0.217			
12. *P. fabricii* (Canada; HM405487)	0.070	0.070	0.070	0.070	0.070	0.068	0.070	0.220	0.223	0.125	0.230		
13. *P. koehleri* (Antarctica; HM196638)	0.260	0.260	0.260	0.260	0.257	0.256	0.260	0.280	0.133	0.259	0.259	0.253	

K2P, Kimura-2 parameter; COI, cytochrome c oxidase subunit I.

width = 2.0–3.4 mm) but *P. phantapus* has small calcified scales (length = 1.0–2.8 mm, width = 0.7–2.0 mm).

A total of 658 bp of mitochondrial COI gene was obtained for the first time from a Korean specimen and was registered in GenBank (Genbank accession number: MF347379). The Korean *P. phantapus* data was comparable to *P. phantapus* data of NCBI (Table 1). The intraspecific pairwise distance value of *P. phantapus* was 0.000–0.003, and there were zero to three nucleotide variations between the Korean specimen and the Atlantic specimens recorded at NCBI (Russia and Canada). This sea cucumber is newly recorded for Korea, based on the morphological and molecular evidence. Thus, two species of genus *Psolus* are recorded from the East Sea of Korea.

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REFERENCES

Ayres WO, 1851. Descriptions of Holothuria. Proceedings Boston Society Natural History, 4:6-148.

Bell FJ, 1882. Studies in the Holothuroidea. I. On the Genus *Psolus* and the forms allied thereto. Proceedings of the Zoological Society of London, 50:641-650.

Corstorphine EA, 2010. DNA barcoding of echinoderms: Species diversity and patterns of molecular evolution. MSc thesis, University of Guelph, Guelph, Ontario.

Deichmann E, 1941. The Holothurioidea collected by the Velero III during the years 1932 to 1938. Part I, Dendrochirota, Allan Hancock Pacific Expeditions, 8:61-196.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R, 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology, 3:294-299.

Gray JE, 1848. Description of a new species of Anatifa. Proceedings of the Zoological Society of London, 16:1-44.

Grube AE, 1840. Aktinien, Echinodermen und Würmer des Adriatischen und Mittelmeeres. Königsberg, pp. 33-43.

Hansson HG, 2001. Echinodermata. In: European Register of Marine Species. A check-list of the marine species in Europe and a bibliography of guides to their identification (Eds., Costello MJ, Emblow CS, White R). Patrimoines Naturels, Paris, pp. 1-463.

Hebert PDN, Cywinska A, Ball SL, deWaard JR, 2003. Biological identifications through DNA barcodes. Proceeding of the Royal Society B: Biological Sciences, 270:313-321. https://doi.org/10.1098/rspb.2002.2218

Hoareau TB, Boissin E, 2010. Design of phylum-specific hybrid primers for DNA barcoding: addressing the need for efficient COI amplification in the Echinodermata. Molecular Ecology Resources, 10:960-967. https://doi.org/10.1111/j.1755-0998.2010.02848.x

Jaeger GF, 1833. De Holothuriis. Dissertatio Inauguralis, Turici, pp. 1-40.

Kimura M, 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 16: 111-120. https://doi.org/10.1007/BF01731581

Lampert K, 1885. Die Seewalzen. Holothurioidea. Eine Systematiche Monographie. In: Reisen im Archipel der Philippinen (Ed., Semper C). Vol. 4. Wissenschaftliche Resultate, Wiesbaden, pp. 1-312.

Layton KKS, Corstorphine EA, Hebert PDN, 2016. Exploring Canadian echinoderm diversity through DNA barcodes. PLoS ONE, 11:e0166118. https://doi.org/10.1371/journal.pone.0166118

Lee T, 2011. A systematic study of Korean Echinoids based on morphology and molecular phylogeny. MS thesis, Sahmyook University, Seoul, Korea, pp. 1-95.

Lütken CF, 1857. Oversigt over Grönlands Echinodermer. Videnskabelige Meddelelser fra den Naturhistoriske Forening i Kjöbenhavn, 1857:1-55.

Mortensen T, 1927. Handbook of echinoderms of British Isles. Oxford University Press, London, pp. 350-448.

Müller OF, 1788. Zoologica Danica seu Animalium Daniae et Norwegiae rariorum ac minus notoruum. Descritiones et Historia, 2:1-55.

O'Loughlin PM, Paulay P, Davey N, Michonneau F, 2011. The Antarctic region as a marine biodiversity hotspot for echinoderms: diversity and diversification of sea cucumbers. Deep-Sea Research Part II: Topical Studies in Oceanography, 58:264-275. https://doi.org/10.1016/j.dsr2.2010.10.011

Paulay G, 2017. Holothuroidea [Internet]. World Register of Marine Species, Accessed 23 May 2017, <http://www.marinespecies.org>.

Pawson DL, 1964. The Holothuroidea collected by the Royal Society expedition to Southern Chile, 1958-1959. Pacific Science, 18:453-470.

Ratnasingham S, Hebert PDN, 2007. BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). Molecular Ecology Notes, 7:355-364. https://doi.org/10.1111/j.1471-8286.2007.01678.x

Rho BJ, Shin S, 1986. A systematic study on Holothuroidea in Cheju-do. Korean Journal of Zoology, 29:245-256.

Selenka E, 1867. Beiträge zur Anatomie und Systematik der Holothurien. Zeitschrift für Wissenschaftliche Zoologie, 17:291-374.

Shin S, 2012. Invertebrate fauna of Korea, Vol. 32, No. 4. Sea cucumber: Echinodermata: Echinozoa: Holothuroidea. National Institute of Biological Resources, Incheon, pp. 1-127.

Shin S, Rho BJ, 1996. Illustrated encyclopedia of fauna and flora of Korea, Vol. 36. Echinodermata. Ministry of Education of Korea, Seoul, pp. 1-780.

Southward EC, Campbell AC, 2006. Echinoderms: keys and notes for the identification of British species. Synopses of the British fauna (new series), 56. Field Studies Council, Shrewsbury, pp. 1-272.

Stimpson W, 1853. Synopsis of the marine Invertebrata of Grand Manan: or the region about the mouth of the Bay of Fundy, New Brunswick. Smithsonian Contributions to Knowledge, 6:1-67.

Strussenfelt AM, 1765. Beskrifning pa er Sjö-krak, Haf-Spöke kaladt. Kon Svenska VetenskAkad, 26:256-266.

Verrill AE, 1866. On the Polyps and Echinoderms of New England, with descriptions of new species. Proceedings of the Boston Society of Natural History, 10:333-375.

Ward RD, Holmes BH, O'Hara TD, 2008. DNA barcoding discriminates echinoderm species. Molecular Ecology Resources, 8:1202-1211. https://doi.org/10.1111/j.1755-0998.2008.02332.x

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